

## CLAIMS

We claim:

5 1 A composition comprising:

- a) a substrate with a surface comprising discrete sites; and
- b) a population of microspheres distributed on said sites.

2 A composition according to claim 1 wherein said sites comprise wells.

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3 A composition according to claim 2 wherein said substrate is a fiber optic bundle or array comprising individual fibers, and said wells are at a first terminal end of said bundle or array.

4 A composition according to claim 2 wherein said substrate is selected from the group  
15 consisting of glass and plastic.

5 A composition according to claim 1 wherein said sites comprise chemically functionalized sites.

20 6 A composition according to claim 1 wherein said microspheres comprise bioactive agents.

7 A composition according to claim 6 wherein said bioactive agents comprise nucleic acids.

8 A composition according to claim 6 wherein said bioactive agents comprise proteins.

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9 A composition according to claim 8 wherein said proteins are selected from the group consisting of enzymes and antibodies.

10 A composition according to claim 1 wherein said population of microspheres comprises  
30 at least a first and a second subpopulation, wherein the microspheres of said first

subpopulation of microspheres are a different size than the microspheres of said second subpopulation.

11 A composition according to claim 3 wherein the individual fibers of said bundle have  
5 substantially the same cross-section.

12 A composition according to claim 3 wherein the individual fibers of said bundle have different cross-sections.

10 13 A composition according to claim 1 wherein said population of microspheres comprises at least a first and a second subpopulation comprising:

- i) a a first and a second bioactive agent, respectively; and
- ii) a first and a second optical signature, respectively, capable of identifying said bioactive agent.

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14 A composition according to claim 13 wherein said at least one of said optical signatures comprises at least one chromophore.

15 A composition according to claim 13 wherein said at least one of said optical signatures  
20 comprises at least one fluorescent dye.

16 A composition according to claim 15 wherein said fluorescent dye is entrapped within said microspheres.

25 17 A composition according to claim 15 wherein said fluorescent dye is attached to said microspheres.

18 A composition according to claim 13 wherein said optical signature comprises at least two fluorescent dyes.

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19 A method of determining the presence of a target analyte in a sample comprising:

a) contacting said sample with a composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation each comprising:

1) a bioactive agent; and

2) an optical signature capable of identifying said bioactive agent;

wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres; and

b) determining the presence or absence of said target analyte.

20 A method according to claim 19 wherein said substrate is a optical fiber bundle and said microspheres are located within wells at a first terminal end of said bundle.

21 A method according to claim 19 further comprising identifying the location of each bioactive agent on said substrate.

22 A method of making a composition comprising:

a) forming a surface comprising individual sites on a substrate;

b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulations each comprising:

i) a bioactive agent; and

ii) an optical signature capable of identifying said bioactive agent.

23 A method according to claim 22 wherein said distributing comprises serially adding said subpopulations to said sites.